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**Interdependent Microcosms for the
Assessment of Pollutants in the
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INTERDEPENDENT MICROCOSMS FOR THE ASSESSMENT OF POLLUTANTS IN THE MARINE ENVIRONMENT†

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Laboratory microcosms are described for assessing the fate and effects of pollutants in marine and estuarine environments. These systems focus on specific ecosystem processes and interactions and are interdependent in that the results of all are necessary for a complete description of a pollutant's environmental impact. The following individual systems are described using methyl parathion as the pollutant: Environmental Fate Screening System; Eco-Core System; Continuous Flow Systems; Aquatic Gradient Avoidance Response System; Benthic Bioassay System.

INTRODUCTION

Traditionally, the effect of a pollutant on the environment has been estimated from the results of toxicity tests using individual species. These tests emphasize the direct effects of pollutants on various life stages of aquatic organisms. In order to provide a better description of the impact of pollutants on estuarine environments, reproducible laboratory systems, or microcosms, that isolate specific physical and biological components are being developed. Using these systems, the effects of pollutants on the interactions and processes which occur in estuarine ecosystems may be determined.

Several types of tests are being developed, ranging from systems which allow investigation of the fate of pollutants in the estuarine environment, to those which focus on the effect of the pollutants on specific behavioral processes. The fate of a pollutant is important because its availability and chemical form as mediated by such processes as volatilization, sorption, metabolism, and abiotic transformation, influences the pollutants' toxicity to aquatic organisms. The insecticide methyl parathion, for example, degrades in the environment into a number of other chemicals with different toxicities and fates (Figure 1). The Environmental Fate Screening System can help delineate processes that influence the movement and transforma-

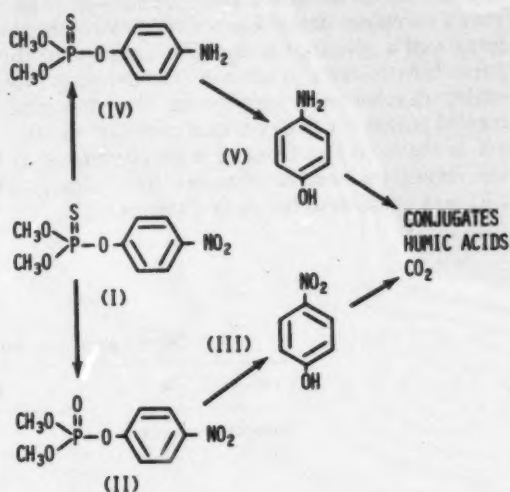


FIGURE 1 Proposed pathway for the breakdown of methyl parathion (I) in aquatic systems. Compounds: methyl paraoxon (II), p-nitrophenol (III), amino methyl parathion (IV), p-aminophenol (V).

tion of pollutants. Utilizing this information, large and small scale Continuous Flow Systems have been developed to characterize and evaluate the degradative potential of indigenous microorganisms and the effects of the chemical on microbial ecology as well as the effects of other biota on the fate of the chemical and the environment.

Behavioral tests allow the study of subtle sublethal effects not revealed in acute bioassay experiments. Two microcosms have been developed which utilize behavioral changes as an indicator of

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chemical exposure effects. The Aquatic Gradient Avoidance Response System allows the quantification of the avoidance responses of estuarine organisms to different pollutant concentration gradients. The Benthic Bioassay System utilizes changes in sediment surface features produced by the lugworm *Arinicola cristata* to monitor the effect of pollutants on the behavior of this organism. The results obtained from both of these systems will provide an indication of pollutant effects at levels far below lethal concentrations and thereby will enable a more environmentally realistic assessment to be made of the potential impact of a pollutant to an estuarine environment.

The tests described are interdependent in that not only are the results of the fate tests used to establish the parameters of the effects tests, but the results from all the microcosms are needed to provide a complete description of the environmental impact of a given pollutant. The purpose of this paper is to describe in some detail the microcosms under development and, using the insecticide methyl parathion as a common pollutant of interest, to show, (i) how these tests are conducted, (ii) the results that can be obtained from them, and (iii) how these results can be interpreted.

MICROCOSMS

a. Environmental Fate Screening System

The Environmental Fate Screening System was designed to determine ecosystem substrate exchange coefficients and rates of pollutant alteration. Environmental factors that influence pollutant fate are: sediment type (sorption processes: clay, organic), water quality (salinity, Eh, pH), physical forces (temperature, sunlight, volatilization), and biological factors (microbiological processes: metabolism, degradation).

This system consists of stoppered 125 ml Erlenmeyer flasks into which air or nitrogen is introduced. XAD-4† resin in the gas exit is used to trap volatile compounds. The small system size facilitates replication and examination of different environmental substrates and processes and allows sequential sampling for determination of transport and transformation rates. Radioisotopes are used to minimize involved analysis and to facilitate simulation of environmental pollution levels.

† Mention of trade names or commercial products does not constitute endorsement by the U.S. Environmental Protection Agency or recommendation for use.

TABLE I
Methyl parathion: percentage^a radioactivity distribution

Day	W	Se	Sc	Pe	Pc	R	Total
Non-sterile systems							
0	67.3	0.9	0.1	22.6	5.6	0	96.5
4	30.7	0.7	2.2	6.5	48.5	0.3	88.9
7	28.2	0.5	1.1	5.4	55.4	0.2	90.6
11	27.4	0.4	1.8	3.9	55.2	0.3	89.0
14	20.5	0.3	2.3	2.9	63.7	0.1	89.8
18	18.8	0.2	2.3	2.7	64.2	0.3	88.5
21	19.4	0.3	2.4	2.2	64.5	0.3	89.1
25	18.3	0.3	1.6	2.5	63.9	0.3	86.9
Sterile systems							
0	68.8	0.9	0.1	29.3	0.7	0	99.8
7	46.9	1.5	0.2	45.1	2.9	0.2	96.8
14	49.0	0.9	0.2	33.7	4.8	2.2	90.8
21	50.0	1.3	0.3	30.8	5.1	3.4	90.9

^a100% = 8.8 µg = 2,649,100 dpm.

W—Water.

Se—Non-suspendable sediment extract.

Sc—Non-suspendable sediment combustion.

Pe—Suspendable particulate extract.

Pc—Suspendable particulate combustion.

R—Volatilization.

In the analytical procedure, the system is fractionated into water, suspendable particulate, and heavier, non-suspendable sediment or sand. These fractions are extracted separately with organic solvent and analyzed by thin layer chromatography/autoradiography, with confirmational analysis by gas or liquid chromatography. The extracted sand and particulate fractions are combusted at 900°C to liberate $^{14}\text{CO}_2$ for determination of unextractable or bound residues. Since all routes of transport from the systems are sampled and all system components are subjected to a total analysis, a materials balance may be calculated for quality control of the data.

Using sterile and unsterile systems, the microbiological component of a typical salt marsh ecosystem was studied with respect to the movement and alteration of the insecticide methyl parathion (Table I). The water compartment (W; 80 ml) in the unsterile systems demonstrated rapid degradation to amino methyl parathion and unextractable products. After 14 days, the parent compound was undetectable. The radioactivity in these systems moved quickly to the detrital particulate fraction (Pc; 2 gm dry weight) where it became irreversibly bound; volatilization (R) and interaction with non-suspendable sediments (S; 80 gm dry weight) were of minor importance in the fate of this chemical. Sterile systems displayed little trans-

formation of methyl parathion; the chemical was either in the water column or extractable from the suspendable particulate (Pe). These data suggest microbial mediation in the irreversible binding of methyl parathion to the suspendable particulate fraction of a salt marsh ecosystem. This phenomenon has been reported previously for agricultural soil¹ and may be of general occurrence for many organophosphorus insecticides.²

Due to the static nature and small size of this system, further validation of these results is required. The system is, however, a valuable screening tool because of its simplicity.

b. Eco-Core System

The main agents for returning organic carbon compounds to the carbon cycle are bacteria and fungi. These organisms affect mineralization of compounds of natural occurrence and might be expected to degrade those of synthetic origin. Therefore, microbial considerations are essential in the proper functioning, response, and recovery of natural and artificial ecosystems for chemical perturbations. Biodegradation potential of any xenobiotic is best predicted for a given environment from information obtained using indigenous microorganisms and their associated environmental conditions.

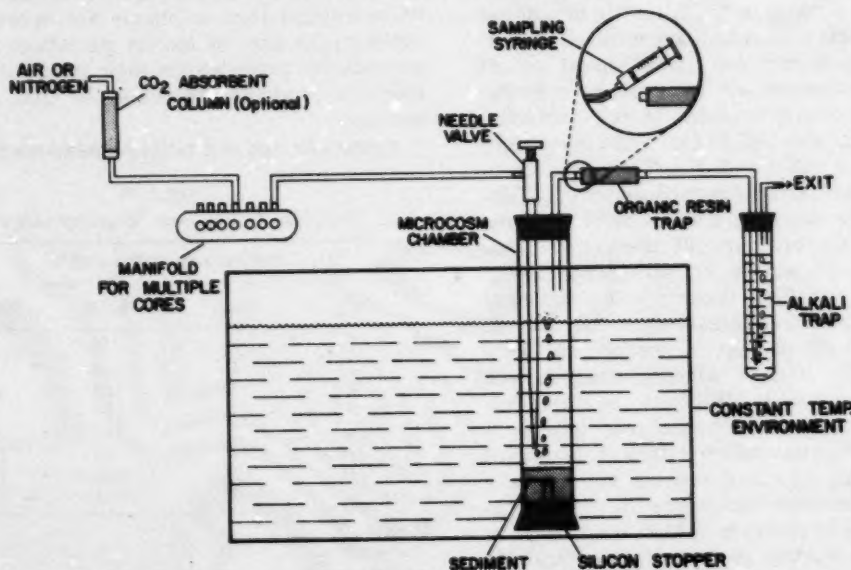


FIGURE 2 Schematic diagram of Eco-Core microcosm.

TABLE II
Metabolism of methyl parathion in a salt marsh Eco-Core

Day	Percentage of MPS remaining ^a			Percentage of polar products		
	Sterile	Sediment	Water	Sterile	Sediment	Water
0	100	100	100	0	0	0
2	92	80	85	4	8	3
4	85	57	66	6	13	7
8	84	15	27	11	23	9
14	84	4	15	16	40	10
31	82	0.01	6	21	54	9

^aMPS added = 1 mg/l containing 22.2 μ Ci.

The Eco-Core is an artificial laboratory microbial microcosm that closely mimics natural conditions for studying microbial interactions. The system evaluates the degradative potential of indigenous microorganisms and the effects of chemical perturbation on microbial ecology. The system (Figure 2) utilizes sterile glass cylinders to extract intact sediment and water cores from the environment. A number of cores can be run simultaneously as described elsewhere.³ Radiolabeled pollutants are used to facilitate analysis. Water column degradation products are continuously monitored by thin-layer chromatography and autoradiography. Volatile products, including $^{14}\text{CO}_2$ and organics, are continuously scrubbed from the air exiting the systems. Effects of the toxicant on the microbial ecology of the cores are monitored by heterotrophic plate counts and total CO_2 evolution. Changes in microbial physiological indices induced by pollutants are obtained by selective media plating of samples from the water column.

Methyl parathion (MPS) fate and effects were examined in a salt-marsh Eco-Core relative to formalin-treated and unamended controls. Table II shows the disappearance of MPS from the water column for three types of cores: (1) with sea water (water), (2) with water and sediment (sediment) and, (3) sterilized with water and sediment (sterilized). The sterile cores showed only a slight decrease with 82 percent of the original MPS extractable after 30 days. All other cores showed rapid disappearance of MPS from the water column. Radioactivity in all sediment cores decreased little after 20 days incubation; however, analysis of the radioactivity indicated only the presence of a major degradation product, amino-methyl parathion (AMPS). The decrease in MPS concentration in water cores was due primarily to adsorption to the glass. Degradation of MPS was considerably

less in water cores than in those cores with sediment.

Polar products (Table II) represented the organic solvent-unextractable radioactivity in the water column and occurred to the greatest extent in the cores with sediments. Production of $^{14}\text{CO}_2$, another indication of aromatic ring cleavage in the parent molecule (MPS), occurred to a greater extent in the sediment cores than in the cores with water only (Table III). No significant $^{14}\text{CO}_2$ evolved from the sterile systems.

Total recovery of the radiolabel amounted to greater than 90 percent in all cases. Combustion of the extracted sediments to $^{14}\text{CO}_2$ showed seventeen percent of the radioactivity to be unextractable from the non-sterile sediment systems. It may be postulated that sediments are necessary for rapid metabolism of methyl parathion and that degradation proceeds through AMPS to unextractable products and finally to CO_2 and cell biomass.

Generally, the microbial populations remained

TABLE III
Evolution of $^{14}\text{CO}_2$ from ^{14}C -methyl parathion

Day	Radioactivity (DPM $\times 10^3$)		
	Sterile ^a	Water	Sediment
0	0	0	0
2	0	0	2
4	8	113	1520
8	3	133	910
14	5	380	830
24	4	716	1956
31	2	990	1550
34	2	554	789
% total dpm added	0.02	3	6

^aWater and sediment treated with 2% formalin.

stable throughout the incubation periods. Data on physiological indices indicated that MPS degradation was accompanied by increases in those organisms possessing phospholytic activity, suggesting this organophosphate insecticide was stimulatory to these organisms. However, the changes in populations were transitory, probably correlating with the disappearance of the parent compound (P-O-C bond). The data suggest the development of an adapted population consistent with the degradation of the pollutant in the core.

As a technique for assessing biodegradation of xenobiotics utilizing natural assemblages of microorganisms, the Eco-Core system can determine: (1) interactive processes contributing to the metabolism of the test compound, (2) changes in microbial populations induced by the toxicant, and (3) ease of handling and system replication. The core is, however, a static system with a disadvantage of possible artifactual changes induced by accumulating metabolites. Reaction rates may be better obtained from flowing systems that resemble the dynamic nature of an aquatic system.

c. Continuous Flow Systems

The application of continuous flow systems to microcosm studies allows the dynamic nature of an ecosystem to be investigated in the laboratory.⁴ Continuous flow systems provide information on the rate and extent to which a pollutant will (a) move into the various compartments of an aquatic ecosystem and (b) be transformed by biological and nonbiological forces. Ideally, the simplest kinetic information will come from a system in which a particular process under study is rate-limited by as few factors as possible. Although this is somewhat unreal in terms of a natural aquatic environment, it does allow determination of which kinetic factors are the most important and how they interrelate with an overall more complex process.

d. Small Scale System

A small scale continuous flow system (500 ml reactor vessel volume) was used to study the fate of methyl parathion under conditions typical of an

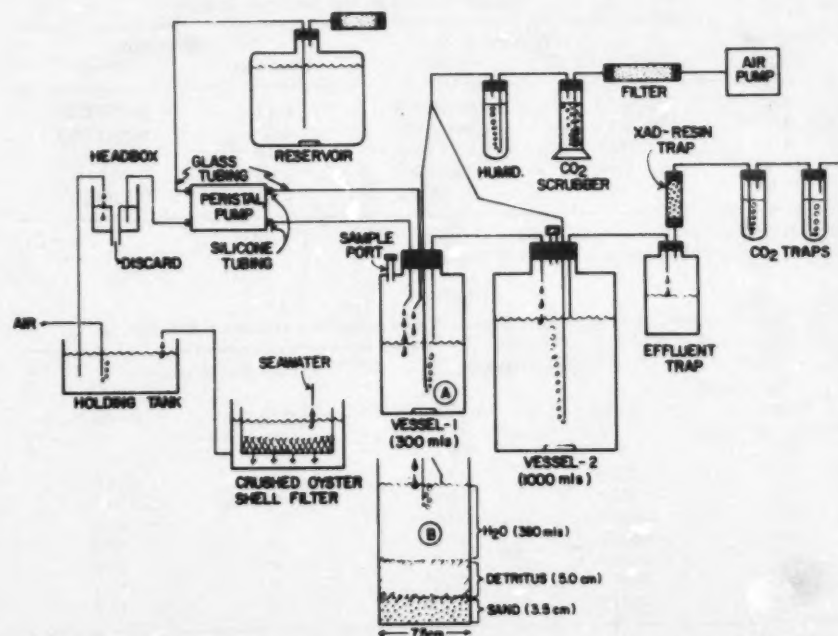


FIGURE 3 Schematic diagram of continuous flow microcosm.

aquatic estuarine environment. This system (Figure 3) is designed with multiple stages, to allow the transformation processes to be studied as a function of dilution. It uses a continuous input of filtered seawater and artificial seawater supplemented with methyl parathion (50–500 ppb). Again, a radiolabeled substrate was used to monitor the rate processes. Transformation products were quantitated by thin layer chromatography, autoradiography, and gas chromatography. Radiolabeled MPS was added to the system either as a spike into the primary reactor vessel (to monitor wash out kinetics) or continuously from a reservoir (where its quasi-steady kinetics were followed).

Table IV shows the stability of MPS in sterile and unsterile continuous flow systems without sediment. No chemical or biological degradation of the substrate occurred. This stability held true under a variety of environmental conditions (different flow rates, nutrient concentrations, seawaters and organic supplements). However, the stabil-

ity of MPS was much less when unmixed sediment was added to the system (see Figure 3). Aeration was adjusted to give an aerobic water column and an anaerobic sediment-water interface. Table V shows that MPS stability, as indicated by CO_2 and polar product production in the unsterile system, decreased with time. This presumably reflects the enrichment of an MPS degrading population of bacteria. MPS was also transformed in the sterile control but not to the same extent. Amino methyl parathion (AMPS) was the principle transformation product in both systems, but *p*-nitrophenol was also detected. The presence of sediment, therefore, promotes the transformation of MPS in estuarine seawater environments. Calculations from these initial experiments indicate a half life of MPS of 40–50 hours for the conditions tested. If methyl parathion input is cut off to the continuous flow system (but maintaining seawater flow), residual MPS in the sediment (shown in the other systems to be tightly bound) slowly releases with time.

TABLE IV
Stability of methyl parathion in continuous flow systems without sediment

Retention volume ^a	Concentration of methyl parathion (mg/l)			
	Sterile control		Unsterile system	
	Reservoir	Vessel	Reservoir	Vessel
1.3	1.10	0.97(81%) ^b	0.97	0.92(94%)
4.2	1.23	1.06(85%)	1.07	0.89(83%)
8.3	0.87	0.97(90%)	1.10	1.04(94%)
11.3	1.08	0.92(85%)	1.03	0.90(87%)
23.6	1.15	1.01(88%)	0.94	0.83(88%)

^aTurnover of one retention volume requires 11 hours.

^bNumbers in parentheses are percentage MPS recovered.

TABLE V
Transformation of ^{14}C -methyl parathion in continuous flow systems with sediment

Hours	Unsterile system		Sterile system ^a	
	$^{14}\text{CO}_2$ ^b	Polar products ^c	$^{14}\text{CO}_2$	Polar products
48	374	7.2	0	5.7
72	2720	15.3	63	8.6
96	3200	13.1	0	10.0
120	6952	22.1	96	11.4
144	6903	27.3	0	16.0
168	17608	30.7	0	16.0
192	23079	32.2	0	15.1
216	26314	31.8	0	15.8

^aTreated with 2 percent formalin in reservoir and sediment.

^b $^{14}\text{CO}_2$ (expressed as dpm) in alkaline traps per day.

^cPercentage of total radioactivity in water column not extractable with methylene chloride.

e. Large Scale Systems

Large continuous flow systems (40 liters reactor vessel volume) are designed to study the fate of a pollutant as affected by marine macrobiota. A vessel containing 30 liters of seawater and a 9 cm thick layer of marsh sediment was used with ^{14}C methyl parathion. The systems were continuously fed filtered seawater, with all water exiting through polystyrene resins. As toxicant movement into the sediment was the major transport process, sediment burrowing lugworms (*Arenicola cristata*) were added to the reactor vessels. The lugworms caused a rapid dispersal of radioactivity throughout the sediment column (Figure 4), compared to a control system with no lugworms. Although these polychaetes enhanced the movement of radioactivity from the water column to the sediment, they never accumulated radioactivity in their tissues. A steady, decreased budget of radioactivity in the systems suggested accumulation of unextractable residues in the sediment, a process verified by the other systems discussed. Analysis of extractable radioactivity indicated both *p*-nitrophenol and amino methyl parathion as degradation products. The amount of degradation was not affected by lugworms but was the result of microbial activity.

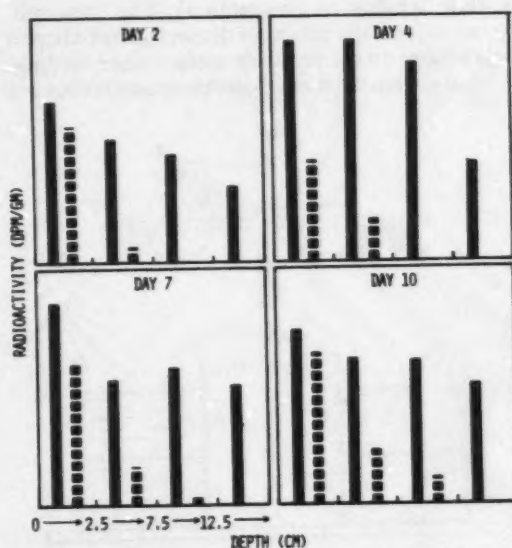


FIGURE 4 Methyl parathion: Radioactivity movement into sediment—lugworm system (solid bar) and control system (broken bar).

These studies demonstrate the application of continuous flow systems to environmental research. These systems are improved tools for studying fate processes because many of the environmental factors which influence fate can be controlled and monitored to a much greater extent than in static systems.

The selective nature of continuous flow systems permits the study of many complex biological processes separated into their component parts. Rates of transformation and compartmental exchange, critical to any fate study, can be obtained from continuous flow studies and applied to environmental situations with greater confidence than similar information from static tests.

f. Aquatic Gradient Avoidance Response System

Behavioral tests allow the study of subtle, sublethal effects which may not be revealed in acute bioassay experiments. Although behavioral processes are complex in nature and many may be affected by toxicants, it is not necessary to know the exact physiological or biochemical mechanism affected if the behavioral change results in an animal being unable to survive in its environment.

The ability to sense and avoid a potentially toxic pollutant is of primary importance to organisms. Avoidance data indicate whether an organism will remain in the area of lethal concentration for a period of time. Avoidance of sublethal concentrations may deplete local fisheries near a pollutant source or alter lower trophic level populations on which the fisheries depend, without resulting in a noticeable "kill." The lack of avoidance of lethal concentrations is also important in relating acute bioassay results to a field situation. Thus, avoidance data may significantly supplement results of toxicity tests.

Most avoidance tests have been performed with Y-shaped troughs, rectangular troughs or cylinders. The organism is presented with a choice between a certain toxicant concentration and uncontaminated water and is usually observed for an hour or less. The system described here (Figure 5) produces a pollutant gradient in a continuous flow system, in which the organism can choose between uncontaminated water and three pollutant concentrations within a rectangular trough. The vertical and horizontal positions of the organism are monitored in eight areas of the trough (four upper, four lower) with two pairs of infrared lights and

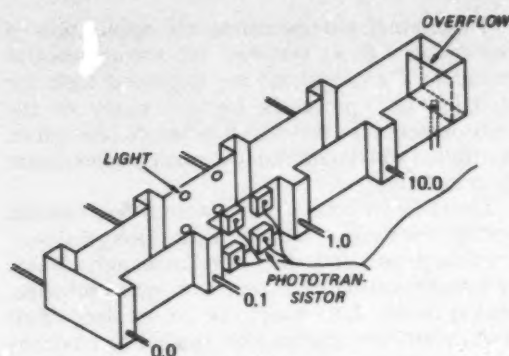


FIGURE 5 Aquatic Gradient Avoidance Response System.

sensors per area. The advantages of this monitoring method have been described elsewhere.⁵ A microprocessor checks each of the eight areas every 0.33 sec. for an organism's presence. Hourly cumulative totals on organism position are collected on teletype tape for processing in a calculator/type reader/X-Y plotter system. Baseline positions are obtained in uncontaminated water, a pollutant gradient is produced, and the time spent in each trough area compared statistically to determine avoidance or attraction.

This system has been utilized to obtain baseline data for several estuarine species. Results of initial tests suggest that some organisms may exhibit a rhythmic presence correlating with photoperiod. Individual pinfish (*Lagodon rhomboides*), for example, were present in the upper four areas during hours of darkness and absent during hours of daylight (Figure 6). Individual blue crabs (*Callinectes sapidus*), however, showed no such

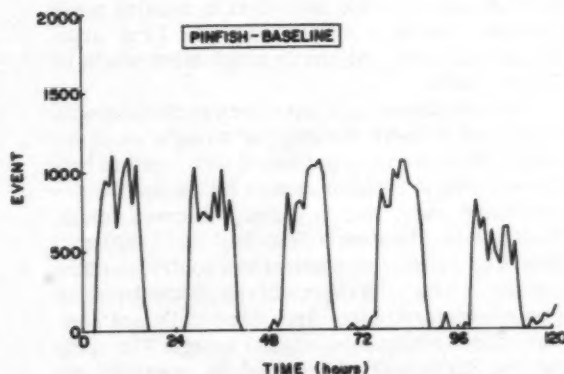


FIGURE 6 Time spent by a pinfish in an upper level position in 0.33 sec/hr events versus elapsed time (hrs) of test.

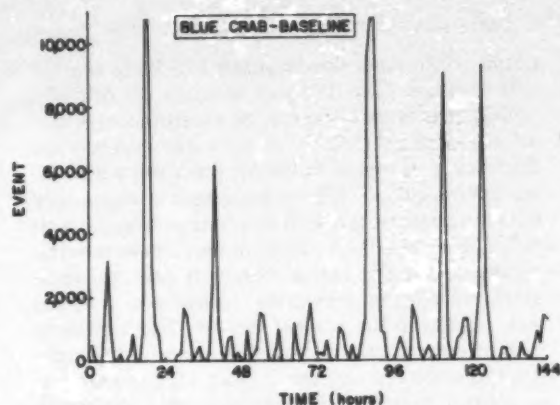


FIGURE 7 Time spent by a blue crab in a lower level position in 0.33 sec/hr events versus elapsed time (hrs) of test.

rhythmicity (Figure 7). Organism groups are also being tested, because group behavior may differ from that of an individual. When baseline information is obtained, aquatic pollutants will be tested. This avoidance data could be useful in assessing effects of pollutants in aquatic environments.

g. Benthic Bioassay System

Many sedentate polychaetes alter a substrate surface as a result of their normal activity (i.e., burrowing, feeding or locomotion). The lugworm, *Arenicola cristata*, produces distinct funnel-shaped depressions on the substrate surface when feeding. A bioassay has been developed to monitor changes

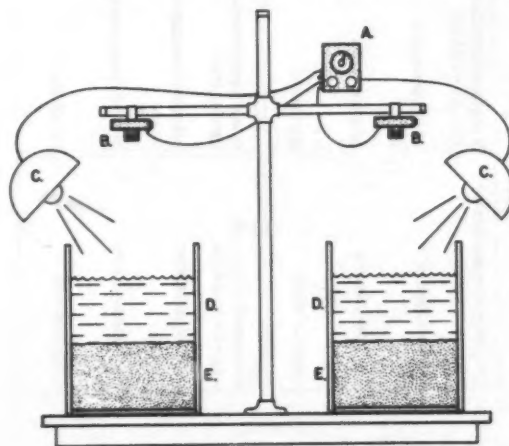


FIGURE 8 Benthic Bioassay System (A = 24-hr. timer, B = 35 mm camera with automatic advance, D and E = 125-l aquaria with 25 cm sand and 75-l seawater).

in surface features produced by lugworms. Rates of feeding formation between exposed and control lugworms are compared in this test.

As reported elsewhere,⁶ tests were conducted in two static, aerated, 125-l tanks containing clean sand and filtered seawater (Figure 8). Six lugworms of similar size were placed in each tank and allowed 48 hours to acclimate. Ground seagrass (*Thalassia testudinum*) was added to both tanks forming a dark mat. The seagrass served as the detrital component of the system and as a source of food. Methyl parathion was introduced into one tank; the undosed tank served as the control. Changes in surface features produced by the lugworms were monitored by time-lapse photography. The feeding funnel area was calculated from photographs taken at 12 hour intervals for 144 hours. Total surface area disturbed with time for both exposed and control lugworms were compared.

Four concentrations of methyl parathion (25, 75, 150, and 215 $\mu\text{g/l}$) were tested. Results are presented in Figure 9. At 25 and 75 $\mu\text{g/l}$ there was no significant difference between rates of feeding funnel formation of exposed and control lug-

worms. However, after 72 hr exposure to 150 and 215 $\mu\text{g/l}$, significant differences in the formation of feeding funnels were observed between exposed and control tanks ($F = 410, 405$, d.f. 2,8 $\alpha = .05$).

Based on these results, a concentration of methyl parathion between 75 and 150 $\mu\text{g/l}$ would cause a decrease in the substrate reworking activity of the lugworm. This could reduce the lugworm mediated exchange between the water and sediment resulting in an increased residence time of the pollutant in the water. Reduced feeding activity of the lugworm could also ultimately result in the death of these organisms. This would affect the overall transport of nutrients and pollutants through the system, as well as alter certain aquatic food webs.

Although the environmental significance of this activity change remains to be fully elucidated, it is clear that this technique can demonstrate a behavioral effect on an important benthic organism at sublethal concentrations. Such a test, which demonstrates effects of low levels of pollutants, will be of value in determining the potential impact of chemicals on the substrate reworking activity of infaunal organisms.

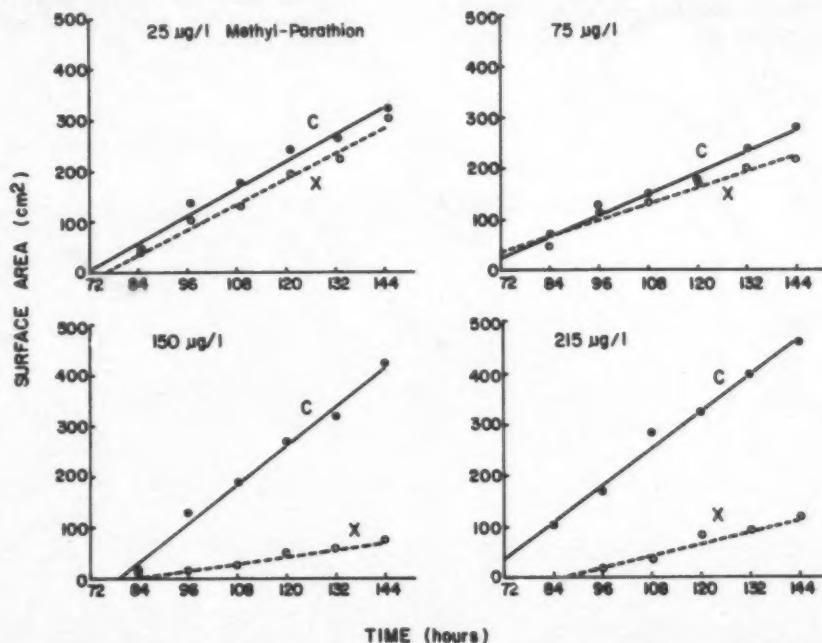


FIGURE 9 Comparison of the rates of surface sediment turned under by exposed (X) and control (C) lugworms at four concentrations of methyl parathion.

CONCLUSIONS

The marine microcosms presented vary in size and analytical methodology requirements. The parameters (transport, transformation, microbial effect, behavioral effect) isolated with these microcosms are important for a more complete understanding of pollutant interactions in the environment. The data presented illustrate the interdependence of these microcosms. The Screening System predicted rapid, irreversible binding of methyl parathion to the sediment. Eco-Core offered a better understanding of the role that microorganisms play in this process and of the pollutant's effect on indigenous bacterial populations. The small continuous flow system demonstrated that the bound chemical was still available for microbial degradation. The large flowing system showed the importance of bioturbation by the benthos in the dissipation of methyl parathion and the benthic bioassay described how this chemical can affect this biophysical process.

These microcosms do not, of course, model all significant environmental processes. The true environmental picture would require the synthesis of these results with data from many additional tests. Integration by computer modeling and validation by field studies will eventually allow better

extrapolation to the natural environment. In the meantime, it is felt that data from microcosms of the type and scale presented will, even without stringent environmental validation, provide regulatory agencies with valuable information for use in decision making.

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